Evaluations of a Ballast Water Treatment to Stop Invasive Species and Tank Corrosion

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ABSTRACT

Invasive species are one of the most destructive environmental problems facing the world today. They can alter habitats, cause local extinction of native species, and have enormous economic impacts. Because ballast water is the primary source of aquatic invasions, the International Maritime Organization has recently passed regulations that will require ocean-going vessels to treat water prior to discharge. It has proven challenging, however, to find an environmentally friendly treatment that is effective at reducing the potential for invasions and yet also acceptable to the shipping industry in terms of safety, time and cost. Our work has focused on evaluations of deoxygenation in general, and Venturi Oxygen Stripping in particular, because of its ability to kill ballast water organisms and to reduce ballast tank corrosion. Results to date suggest that this approach has the potential to be an effective ballast water treatment option.

KEY WORDS: Ballast Water Treatment; Invasive Species; Deoxygenation; Corrosion.

INTRODUCTION

Statement of Problem and Prospectus – Invasions by non-native aquatic species are increasingly common worldwide in coastal habitats (Ruiz et al. 2002a,b) and it is widely accepted that ballast water is the most important vector responsible for transporting and introducing non-native species to new biogeographic regions (Carlton and Geller 1993; Cohen and Carlton 1998). It has proven challenging, however, to find an environmentally friendly ballast water treatment that is effective at reducing the potential for introductions and yet also acceptable to the shipping industry in terms of safety, time, cost, and space requirements. For instance, the offshore exchange of ballast water is currently mandated by U.S. Coast Guard (and other jurisdictions) to reduce introductions, since coastal organisms are unlikely to invade open ocean areas, and vice versa. However, the process can be time-consuming (thus costly), cannot be performed in rough sea conditions, is not appropriate for some shipping routes that do not travel far enough offshore, and has limited effectiveness in some environments and for certain vessel designs (e.g., Cooper et al. 2002; Ruiz et al. in prep.). Because of these reasons, exchange is considered an interim solution that will likely be phased out over the next several years as regulations requiring treatment are implemented.

In the past, the development or identification of feasible and effective ballast water treatments had been hindered in part by the lack of accepted standards. However, the International Maritime Organization (IMO) has recently (February 13, 2004) adopted the Convention for the Control and Management of Ships' Ballast Water that includes ballast water standards. The U.S. Coast Guard and legislative efforts have also made strides towards regulations that will provide standards for technology performance to be measured against. However, for any ballast water management strategy to be successful, the shipping industry must be able to comply (e.g., non-conflicting with other regulations such as those designed for crew, vessel, and environmental safety) and compliance must be measurable. With the recent progress of promising treatments, the shipping industry does appear prepared to adopt technologies that are safe and effective.

Our work indicates that deoxygenation might be such a treatment, with an added benefit of reductions in ballast tank corrosion rates (Tamburri et al. 2002; Tamburri et al. 2003). We have taken a phased approach in the evaluation of deoxygenation as a treatment to prevent the transport on non-native species in ballast water and are now at the stage of field-testing of a prototype system during normal vessel operations. The following is a summary of our work to date and our presentation will focus in particular on results of our ongoing evaluations onboard the active oceangoing vessels.

PHASE I: PROOF-OF-CONCEPT STUDIES

The vast majority of the world's ships are constructed of carbon steel, which corrodes rapidly when exposed to oxygen and water. The cost to prevent, maintain, and repair corrosion on individual vessels typically runs into the millions of dollars and for the entire U.S. marine shipping industry it is estimate at $2.7 billion annually (Koch et al., 2001). Ballast tank corrosion in particular is considered to be the most important factor leading to age related structural degradation of ships (Paik et al. 2003).

In a study completed in 1998, Sumitomo Heavy Industries found that deoxygenating ballast waters (purging with nitrogen gas to drop oxygen levels to approximately 0.2 mg/l) decreases the rate of uniform corrosion by 90% and represents a significant savings for ship owners when compared to other corrosion prevention approaches currently available (approximately $80,000/year/vessel saved when compared to the standard painting and maintenance; Matsuda et al. 1999). These results are supported by the anecdotal observations of the Hellespont Group, who state that corrosion in ballast tanks on their tankers has been "completely arrested" after the addition of low-sulfur inert gasses, which produces hypoxic conditions. Continuous hypoxia has also been suggested as a way to reduce microbial induced corrosion in other marine applications, such as pipe systems (Lutey 2001; Pope and Pope 2001).

To test whether deoxygenation may also limit invasion, we carried out laboratory oxygen tolerance experiments on the larvae of three widely introduced aquatic nuisance species (Australian tubeworm *Ficopomatus enigmaticus*, European zebra mussel *Dreissena polymorpha*, and European green crab *Carcinus meanas*) using oxygen levels comparable to those in the Sumitomo shipboard corrosion study (< 0.8 mg/l). Significant levels of mortality were found in nitrogen treated water after only two or three days (Tamburri et al. 2002). Two separate literature reviews of oxygen tolerance for various aquatic species further support the conclusion that few organisms will be able to withstand extended periods of exposure to deoxygenated ballast water. For example, by far the most abundant animals found in ballast water are copepod crustaceans (Carlton and Geller 1993; Smith et al. 1999) and shallow water and estuarine species that are unable to withstand 24 hours of exposure to hypoxia (e.g., Roman et al., 1993; Lutz et al. 1994; Stalder and Marcus 1997).

Small plant and algal parts (fragments, spores, and seeds) as well as single-celled phytoplankton, protoctists, fungi, and bacteria are also often transported in ballast water (e.g., Hallegraeff and Bolch 1991; Carlton and Geller 1993, Wonham et al., 2000). These microscopic components of ballast water have not been thoroughly characterized. However, it appears from our reviews that their tolerances for low oxygen environments will vary greatly. There are examples of species that are very sensitive to hypoxic conditions (e.g., filamentous fungi, Padgett et al. 1989; zoospores of the seaweed *Undaria pinnatifida*, Mountfort et al. 1999), as well as counter-examples of species that can withstand low oxygen levels (e.g., resistant cysts of some dinoflagellates, Hallegraeff 1998). Marine bacteria, in particular, will have dramatically different responses to the conditions created by simply sparging with nitrogen. While most obligate aerobic strains will be unable to grow over extended periods of hypoxia, some facultative and obligate anaerobic bacteria may actually thrive under the conditions found in nitrogen treated ballast water. Our previous work therefore concluded that ballast water deoxygenation alone (sparging with nitrogen gas maintaining hypoxia as done by Sumitomo) would likely be highly effective at reducing introductions of aquatic animals (larvae, juveniles, and adults stages) but may have mixed success at eliminating introductions by members of other taxa.

PHASE II: MESOCOSM EXPERIMENTS

Based on initial results, we conducted further evaluations of deoxygenation, focusing on a specially designed ballast water treatment system called Venturi Oxygen Stripping (VOS), to prevent the transport of aquatic species as well as ballast tank corrosion. VOS was developed and patented by NEI Treatment Systems, LLC, a U.S.-based environmental engineer firm. In February 2003, we began our NOAA, Fish and Wildlife Service and MARAD supported project as our second phase with three specific goals:

1) Evaluate the abilities and potential of VOS as a ballast water deoxygenation method,
2) Examine the impact of this oxygen stripping technique on the immediate and long-term survival of natural Chesapeake Bay planktonic organisms, and
3) Quantify corrosion rates and establish the corrosion mechanism under VOS deoxygenated conditions (with particular emphasis on microbiologically influenced corrosion).

Optimizing deoxygenation – A key to the success of deoxygenation as a ballast water treatment is to efficiently maintain levels of oxygen in tanks that both kills the majority of aquatic organisms while also reducing corrosion rates; between 0.2 and 1.0 mg/l. Several deoxygenation methods have been proposed or tested, including nitrogen sparging (described above), vacuums, horizontally placed diffuser plates, and biological processes. These oxygen removal techniques vary in degree of effectiveness (including time required until hypoxic ballast water is produced), associated costs, and logistic constraints. However, a broad survey of options concluded that VOS is currently the most efficient and feasible way to remove oxygen from ballast water onboard vessels. Therefore, beginning in 2003, our research into deoxygenation to prevent aquatic invasions and ballast tanks corrosion has focused on VOS.

VOS is a rapid (< 10 seconds), in-line, single-pass system that mixes inert gas directly into ballast water as it is drawn into the vessel (Figure 1). The inert gas is produced by combusting low-sulfur marine diesel (generating mostly nitrogen with small amounts of carbon dioxide and only trace levels of oxygen) in a device similar to the inert gas generators commonly used on tankers. The gas is mixed with the ballast water using a venturi injector manifold that is installed in-line, just down-stream of the ballast pump. The venturi injectors create a micro-fine bubble emulsion where dissolved oxygen quickly diffuses out of the water into the gas. Because adding carbon dioxide in solution forms both carbonic and carboxylic acid, the pH of treated water is also reduced to between 5.5 and 6. This system is designed so that the same inert gas is also used to blanket all headspaces and the entire ballast tank when empty to maintain permanent hypoxia. Continuously maintaining a deoxygenated environment in ballast tanks appears to be a critical factor for corrosion prevention (Pope and Pope 2001; Lee et al. 2005).

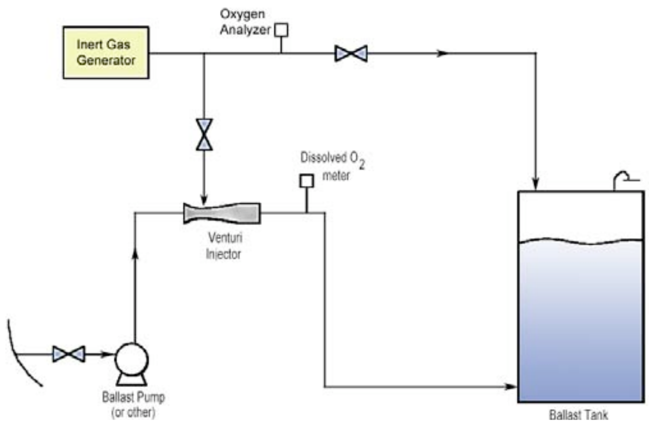

Figure 1. Basic schematic of the VOS system onboard a vessel.

Survivorship of natural planktonic organisms – The mortality rates of planktonic organisms produced by VOS are higher than predicted for our laboratory scale nitrogen sparging experiments and published literature on the oxygen tolerances of aquatic organisms (Tamburri et al. 2003). Although it is clear that the hypoxic conditions alone are toxic to the majority of planktonic organisms, it was the combination of reduced oxygen levels (between 0.2 and 1.0 mg/l), CO_2 saturation with lowered pH (between 5.5 and 6.0), and mechanical disruption as organisms pass through the venturi injector that is responsible for the rapid removal of potential invaders. Therefore, VOS is not simply a deoxygenation treatment but a single unit or method that produces a combination treatment.

In dockside, mesocosm experiments at the Chesapeake Biological Laboratory (CBL) in Solomons, MD, the dissolved oxygen levels and pH in the untreated or control 94-liter tanks were between 8.18 – 11.01 mg/l and 7.61 – 8.20 respectively. The dissolved oxygen levels and pH in similar VOS treated tanks dropped to 0.26 – 0.87 mg/l and 5.46 – 5.62 respectively. These changes to the physical environment in the treated tanks led to a greater than 99% mortality of natural zooplankton (copepods, barnacle larvae, polychaete larvae, cladocerans, rotifers, crustacean nauplii, bivalve larvae, and nematodes) in less than 48 hours while the majority of zooplankton survived in the control cylinders (> 50 % survival even after 96 hours holding time). In other words, after 48 hours of exposure to the VOS system, there were only on average 6 (± 9 SD) living individual organisms greater than 50 µm in size per cubic meter and zero after 72 hours. Furthermore, no intact individuals scored as dead after 48 hours recovered after being placed back in aerated water for 24 hours. Therefore zooplankton are not simply narcotized but are being killed.

In addition to hypoxia and lowered pH, many of the larger zooplankton (mostly copepods) also appear to be killed instantaneously by being damaged (Figure 2) as they passed through the venturi injector, which creates large amounts of cavitation and turbulence.

Figure 2. Copepod torn in half by passing through the VOS system.

Our mesocosm experiments also demonstrate that VOS will significantly reduce the abundance of phytoplankton (mixtures of naturally occurring diatoms and dinoflagellates; Tamburri et al. 2003). Because the dockside, mesocosm experiments were conducted in the dark to mimic ballast tank light conditions, our initial measurements of chlorophyll in samples taken from treated and control tanks are difficult to interpret. It is common for algae to resorb photopigments when stressed by low light or other harsh environmental conditions (e.g., Deventer and Heckman 1996). Although greater decreases were found in treated water, our results show a dramatic and rapid decline in total active chlorophyll for both treated and control tanks through time. Another estimate of impacts of VOS on phytoplankton is the number of algal cells per unit volume through time. The main limitation of direct cell counts is that they do not distinguish between live (or viable) and dead cells so will typically overestimate abundances. Through direct counts, we found that while the abundance of phytoplankton did not change significantly in the control tanks over four days, it dropped between 70 - 80% of initial concentrations in the treated tanks. Depending on what time of year the particular trial was conducted, the numbers of cells per ml decreased to only a few hundred after 96 hours. While hypoxia is likely the main factor causing the mortality of zooplankton, CO_2 saturation and pH reduction (5.5 - 6.0) appears to be responsible for the removal of algae (in addition to the obvious absence of light). This is not surprising given the recent review of 21 publications on the response of nearly 40 different species of diatoms and dinoflagellates that concluded a small pH reduction to 6.5 severely affected phytoplankton growth (Hinga 2002).

Results from our dockside, mesocosm experiments also demonstrate that there is no significant change in numbers of total bacteria cells through time or between control and treatment (Tamburri et al. 2003). Although treated ballast water contains elevated levels of organic material (i.e., dead plankton), the VOS system produces a "dead-end" condition of continuous hypoxia. This dissolved oxygen concentration inhibits the growth of aerobic microbes but is still toxic to obligate anaerobic microbes such as sulfur reducing bacteria. In fact, VOS appears to kill several pathogens that are of particular concern in ballast water. Although bacteria such as *Escherichia coli* and *Vibrio cholera* are facultative anaerobes and adapted to acidic conditions found in digestive systems, they are extremely susceptible to rapid drops in pH with 100% mortality after only 30 minutes of exposure to a 2 unit pH decrease (Merrell and Camilli 1999). This could explain the recent result of Husain et al. (2003) which reported a greater than 99% mortality of *V. cholera* after a 24 h exposure to hypoxia and a pH of 5.5. Furthermore, a mixture of low dissolved oxygen (between 0.1 and 1.0 mg/l) and a pH of 5 has also been demonstrated to effectively reduce the levels of *Enterococci* form 7.8 x 10^6 per 100 ml to less than 500 per 100 ml after extended exposure in batch scale experiment (Awuah et al. 2002). We have also tested for this effect directly as a response of VOS (see below).

Corrosion rates and mechanism – Results from our corrosion experiments with the Naval Research Laboratory have confirmed the findings of Sumitomo Heavy Industries (Matsuda et al. 1999). When the VOS system is operated correctly, instantaneous corrosion rates are reduced by 50% to 80% (using IR compensated Linear Polarization Resistance analysis) when compared to the corrosion of steel under normal ballasting conditions (Lee et al. 2005).

Additional evaluations of corrosion rates under control and VOS treatment conditions are also currently underway by BMT Fleet Technology Ltd. This work is being carried out using similar methods employed to investigate deterioration of vessel structural integrity due to chemical treatment of ballast water (commissioned by the Ship Structure Committee). While this analysis of corrosion is still ongoing, initial results further support the conclusion that VOS will dramatically reduce corrosion rates (Figure 3).

Figure 3. The top row is steel plates from a test tank under typical oxygenated seawater conditions after 30 days. The bottom row is steel plates from a test tank with VOS treatment water after 30 days.

The reduction in corrosion as a result of the this deoxygenation system has the potential to decrease ballast tank maintenance. It is possible that the amount of material repair and replacement required during vessel servicing, and the frequency of vessel servicing, can be reduced significantly. Furthermore, with reduced wastage rate of ballast tank steel, ships may remain more structurally sound at advanced ages (Paik et al. 2003).

PHASE III: PILOT-SCALE EVALUATIONS

We extended our work with funding from the Great Lakes Fishery Commission (through the Fish and Wildlife Service) to add an additional key step in the process of evaluating this ballast water treatment system. A pilot-scale VOS system was built by NEI at CBL (Figure 4), where local waters are very productive (high concentrations of plankton) and range seasonally from temperatures of 6 – 28ºC and salinities of 8 – 18 ppt. The system consists of a 1,500 gpm (350 m^3/hr) ballast water pump with 8-inch diameter PVC piping, valves, and venturi injector; a full-scale inert gas generator; and a series of replicate mesocosms enclosed in a small work space. This is the equivalent treatment system for a large-sized cruise ship, but approximately one-tenth scale for a VLCC tanker.

Figure 4. Pilot-Scale VOS system at the Chesapeake Biological Laboratory, Solomons, MD.

Mechanical and engineering evaluations have demonstrated that the VOS system will produce the desired conditions that will cause the mortality of planktonic organisms and that will reduce corrosion rates. Long-term (12 hour) reliability tests have yet to result in any drift in operational performance or mechanical problems.

Results from pilot-scale biological experiments demonstrate that the VOS system alone can meet IMO standards for all three categories (zooplankton, phytoplankton and bacteria) on voyages of more than four days. Figures 5, 6, and 7 show some of our results of biological efficacy of VOS at the pilot-scale.

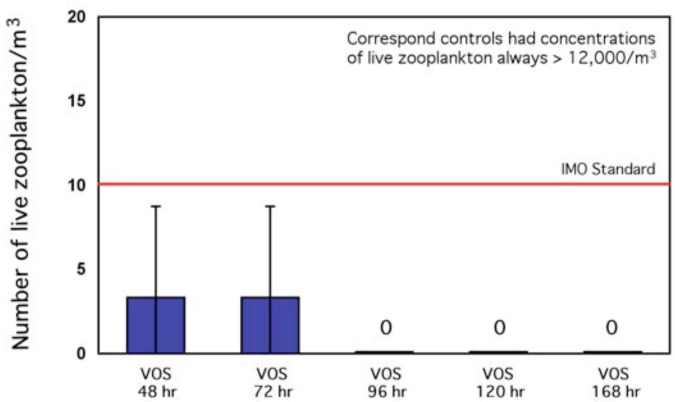

Figure 5. Number of live organisms > 50 µm per cubic meter of water after replicate trial (n = 3) of 2, 3, 4, 5 and 7 days holding times after treatment with VOS at the pilot-scale.

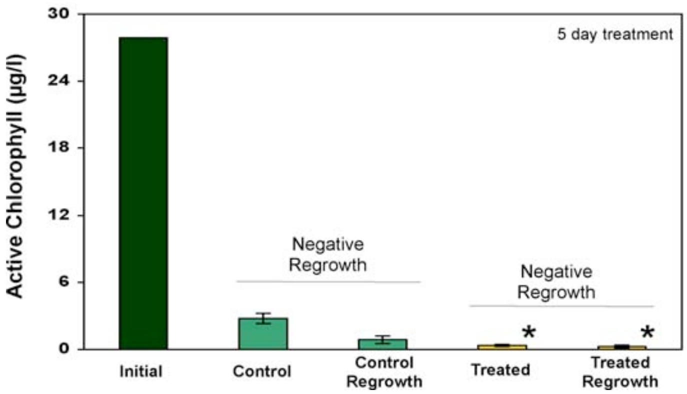

Figure 6. Results from extractive chlorophyll analyses of natural bay water just prior to starting the experiment (Initial, n = 1), untreated water (Control, n = 3), and VOS treated water (Treated, n = 3). Control and treated water were held in darkened mesocosms for 120 hours and active chlorophyll quantified immediately after holding time and after allowed to "regrow" under light and aerated conditions for 24 hour. Chlorophyll in regrowth water was less than water prior to regrowth and below method detection limits (*) indicating essentially no viable phytoplankton after 5 days. Cell counts from fixed samples are still underway but < 4 organisms between 10 and 50 µm in size per ml in VOS treated water were found after 7-day trials (IMO Standard is 10/ml).

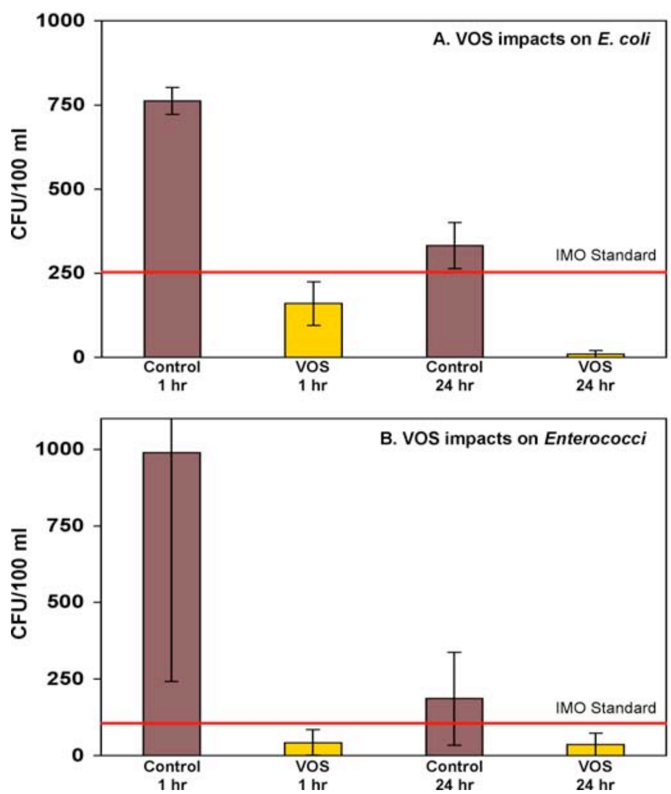

Figure 7A & 7B. Abundances of *E. coli* (A) and *Entrococci* (B) in seeding experiments with control (n = 3) and VOS treated (n = 3) water after either 1 hour or 1 day holding periods without light. In separate experiments, single strains of bacteria were cultured and added to 50 L of natural bay water at approximately 800 cfu/100 ml. The seeded water was pumped into replicate 1 L flasks, with control flasks open to air and treated flask sealed to maintain hypoxic/low pH conditions. One 100 ml subsample from each flask was analyzed for concentrations of culturable *E. coli* or *Enterococci* using a commercially available chromogenic substrate method (IDEXX Laboratories, Inc.; Noble et al. 2003).

We were also able to take advantage of the pilot-scale testing to examine if discharging treated water alters the environment. Periodic measurements of the temperature, salinity, dissolved oxygen, and pH of ambient water (up current > 20 meters from discharge) and at varying distances from the discharge pipe during all pilot-scale evaluations demonstrated that there is almost no measurable change to receiving water. Differences in the physical conditions could only be detected when measurements were taken within 1 meter of the discharge pipe but the slight reductions in dissolved oxygen and pH were still well above harmful levels for aquatic organisms and well above regulation limits (Table 1). These results hold even after 12 hours of continuous treated water release at 350 m^3/hr.

Table 1. Range in values for dissolved oxygen (DO) and pH measured in ambient water and receiving water (< 1 meter of VOS treated water release point). The treated water was discharged at 350 m^3/hr and for as long as 12 hours.

Parameters	Ambient	Receiving
DO (mg/l)	8.7 – 11.3	6.9 – 9.7
pH	7.6 – 7.8	6.7- 7.0

PHASE IV: SHIPBOARD EVALUATIONS

The following is a short summary of a partial and complex dataset. Not all data has been included and additional test voyages, analyses, and statistical evaluations are ongoing.

Engineering Efficacy – NEI has furnished and installed a prototype VOS system for the 40,000 DWT bulk carrier *M/V Pat Cantrell*, operated by TECO Ocean Shipping in U.S. waters of the Gulf of Mexico and Florida's Atlantic coast (Figure 8). This particular VOS system operates at 1,000 cubic meters per hour and has produced the intended ballast water conditions (dissolved oxygen < 1.0 mg/l, pH approximately 6) on three consecutive test voyages, from January through May 2005. Following a fine-tuning process of the prototype to operate optimally onboard the *Pat Cantrell*, all mechanical evaluations (e.g., monitoring ballasting rates and ballast water pressure with and without treatment) to date have shown that it does not impact normal vessel operations.

Water Quality Analysis – In addition to monitoring the specific physical parameters of control and treated ballast water that are altered by VOS (dissolved oxygen and pH), we have also considered other potential impacts to treated ballast water and alterations to harbor water where it is released. For example, analysis of total petroleum hydrocarbons were measured and found to be below detection limits in both control and treated ballast water (Phase Separation Science, Inc., No. 05060316).

Figure 8. Shipboard evaluations are underway on the TECO Ocean Shipping bulk carrier *M/V Pat Cantrell* (top). Twin 12-inch stainless steel venturi injectors as part of the VOS system (bottom left). Biological sampling to determine system efficacy (bottom right).

To date, only one set of measurements has been taken to quantify impacts of VOS treated water on receiving harbor water for this full-scale shipboard study because of vessel logistic constraints. Measurements of dissolved oxygen, pH, temperature and salinity were taken 10 minutes prior to (ambient water) and 15 minutes after the *Pat Cantrell* began discharging VOS treated water at approximately 1,000 m^3/hr. Table 2 below presents dissolved oxygen as mg/l and pH values.

Table 2. The water in ballast tanks prior to release had dissolved oxygen levels below 1.0 mg/l and pH values of approximately 6.
[b] Lowest values recorded in VOS receiving water < 1 meter from the seachest as the instrument was lowered and raised vertically in the water column.
[c] Lowest values recorded in VOS receiving water 3 meters port and starboard of the seachest as the instrument was lowered and raised vertically in the water column.

Date	**Ambient Water**		**Receiving Water < 1 m[b]**		**Receiving Water 3 m[c]**	
	DO mg/l	pH	DO mg/l	pH	DO mg/l	pH
5/12/2005	**6.4**	**7.6**	**5.5**	**7.2**	**6.3**	**7.6**

Biological Efficacy – Three biological test voyages have been completed with two more planned. In all cases, prior to departure, shipside measurements are made and samples collected to characterize the water being brought onboard the vessel (T0) from the St. John's River (Jacksonville, FL). In addition to standard measures of temperature, salinity, dissolved oxygen and pH, we have also been collecting samples for in vivo chlorophyll, total suspended solids (TSS), particulate organic carbon (POC) and the presence of *Vibrio cholerae*.

The biological evaluations are designed to examine the concentrations of organisms before (T1; immediately after ballasting), during (T2; mid-voyage/approx. 2 days) and after a test voyage (T3; prior to discharge/approx. 4 to 5 days) in paired control and treated ballast tanks (two each, four tanks total). This allows both estimates of percent reduction in organism concentration due to VOS treatment by comparison with the control tank and to determine the amount of time required by VOS to meet IMO standards.

Organisms have been divided into the size fractionations and bacterial types described in IMO standards. Zooplankton are exampled in > 50 microns samples that are collected at each time period using a specially designed and calibrated submersible pump/net system. Two replicate 0.5 cubic meter samples are taken near the surface of the ballast tanks and two replicate 0.5 cubic meter samples are taken 1 meter above the bottom of the ballast tanks. For all test voyages, T3 samples (taken just before the vessel arrives in port) are analyzed for live and dead zooplankton under a dissecting microscope prior to fixation. In some case, we also have direct counts of live/dead for T2 samples. While total abundance and taxonomic classification for many samples is still being determined, the live/dead data from the T3 samples have shown reductions in zooplankton after 4 to 5 days. Initial concentrations of zooplankton at T1 in ballast tanks have been remarkably high (in some cases over 500,000 per cubic meter) but as expected, abundances drop through time in both control and treated tanks. However, while several thousand are still living in the control tanks after 4 days, few if any living organism > 50 microns can be found in VOS treated tanks. Table 3 shows examples from two test voyages for the numbers of live zooplankton (mostly copepods, barnacle nauplii and cyprids, trocophores, turbellaria, rotifers, platyhelminthes, and polychaetes) in replicate control and treated tanks after voyages just over four days.

Table 3. The mean and standard deviation of live organisms per m^3 of ballast water > µm 50 microns in size in Control and VOS treated ballast tanks after approximately 100 hours.
* Designates a conservative estimate further analysis is underway.

Date	**Route**	**Control (mean ± SD)**	**VOS (mean ± SD)**
5/12/2005	Jacksonville, FL to Port Arthur, TX	**> 20,000 ***	**0 ± 0**
5/25/2005	Jacksonville, FL to Houston, TX	**11,650 ± 4,454**	**10 ± 9**

Protists between 10 and 50 micron in size are quantified in a similar way using whole water samples. Two replicate 2-l Kimmerer bottle samples are taken in each test tank near the surface and two replicate samples are taken 1 meter above the bottom, at each time point described above. Sub-samples are fixed with standard Lugol's solution and placed in 250ml amber bottles for direct cell counts under a microscope (analysis from the first three test voyages is still underway). Because these direct cell counts cannot distinguish between live and dead cells, we have also been measuring in vivo fluorescence for chlorophyll from the water samples collected from each tank at each time point, and are conducting regrowth experiments. Final samples from each test tank (T3) are measured for in vivo chlorophyll and then placed in flasks (covered but open to air), with algal nutrients, and under grow-lights. Subsequent in vivo chlorophyll measurements are taken from each sample after 12, 24 and 36 hours. To date, we have found increasing chlorophyll concentrations in samples taken from controls but not VOS treated tanks. While a decrease in chlorophyll concentration in regrowth experiments is not definitive proof of no viable phytoplankton, an increase in chlorophyll levels through time does suggest that at least some viable organisms were present in the original T3 samples. Complete analysis of data is still underway.

In addition to quantifying the specific bacteria described in the IMO standards, we are also determining total bacteria concentrations. Sub-samples are taken from the Kimmerer bottles described above, fixed and counted by flow cytometry (analysis is still underway). Additional sub-samples at T3 are also analyzed for concentrations of *E. coli* and *Enterococci* using the chromogenic substrate, quanti-tray most probable number analysis described above. *Enterococci* have yet to be detected at any significant level in test tanks after the vessel arrived in port. *E. coli* has been detected but on only one test voyage to date. Concentrations in control and treated tanks for *E. coli* were both below 100 cfu with fewer in treated than control (although not significantly different). Finally, as mentioned above, *V. cholerae* is also being measured using DFA analysis (New Horizon Diagnostics). During one test voyage (5/25/2005), an average of 25 cells/ml of the serotype 01 were found shipside prior to departure. However, none was found in VOS treated tanks and only one cell (total, not per ml) was found in control tanks at T3.

We now also have funding in place from the NOAA Ballast Water Technology Demonstration Program to extend the scope of performance testing to include a different ocean basin (with different biota) and vessel type than is currently being evaluated and to conduct long-term monitoring of VOS efficacy for an entire vessel's ballast water under routine, continuous operations as part of U.S. Coast Guard's Shipboard Technology Evaluation Program (STEP). This final demonstration will involve a completely automated treatment system and a vessel operating in the Pacific and is critical to determine the ability of VOS to continuously meet regulations under normal vessel operations.

SUMMARY AND CONCLUSIONS

Multiple US and international organizations and agencies rate the invasive species issue as one of the three most destructive environmental problems facing the world today (e.g., United Nation, U.S. Department of State, and Union of Concerned Scientists) and oceangoing vessel ballast water is recognized as the primary source of aquatic species invasions (National Research Council 1996). The newly adopted IMO Ballast Water Convention will eventually require over 50,000 internationally trading vessels to treat ballast water prior to discharge.

Although additional real-world testing is still required, our results to date suggest that Venturi Oxygen Stripping has the potential to be an effective, safe and practical ballast water treatment option. The hypoxia, low pH, and mechanical disruption produced by this system appears to be lethal to organisms found in ballast water, reducing abundances to IMO discharge standards after 4 to 5 days of tank holding time. The VOS system may also directly benefits the shipping industry. The results of our work, and of others, have demonstrated that this system can reduce corrosion rates of ballast tanks between 50% and 90%. This reduction in corrosion has the potential to reduce maintenance cost for ship owners and with reduced wastage rate of ballast tank steel, ships may also remain more structurally sound at advance ages. This ballast water treatment system may therefore represent a rare example of a technology that simultaneously has benefits for the environment and industry.

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